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EVOLUTIONARY SYSTEMATICS

Gordius nixus sp. nov.: first report of a horsehair worm (Gordiida, Nematomorpha) from snow in Pakistan

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Abstract

Gordius nixus **sp. nov.** is described as a new species from Pakistan. It is the first record of a gordiid nematomorph from Pakistan. It was found on snow, which is unusual for nematomorphs. So far, no further life cycle data or host records can be given. The new species resembles other *Gordius* species, but is characterized by a dense covering of spines in the anterior body region, which is unique among the genus *Gordius*.

Key Words

Gordiida, Nematomorpha, Pakistan, snow

Introduction

Comprising about 360 currently described species, the phylum Nematomorpha, commonly known as horsehair worms, is represented both in freshwater (Gordiida) and marine (Nectonematoida) habitats (Poinar 2008; Schmidt-Rhaesa 2013). Nematomorphs are parasitic when immature and free living in adult stages (Hanelt et al. 2005). Marine species in the single genus *Nectonema* parasitise decapod crustaceans while species of the 18 freshwater genera usually parasitise insects including orthopterans, beetles and praying mantids as well as some other arthropods such as centipedes and millipedes (Hanelt et al. 2005; Schmidt-Rhaesa 2013). Gordiids occur worldwide, but reports of their presence are highly uneven. In many regions, they remain poorly documented, including Parkistan, where no species have been recorded to date.

Identification of horsehair worms is largely based on morphological characters. In the genus *Gordius*, males are recognized by the presence of a postcloacal crescent and paired rounded tail lobes. In the only other genus with a postcloacal crescent, *Acutogordius*, the tail lobes are pointed. Other differences between *Gordius* species are often minute and their variability is insufficiently understood, making it sometimes difficult to distinguish within species variation from between species variation (Schmidt-Rhaesa 2010). Female specimens are even harder to identify to species level than males, because they have fewer diagnostic characters. Species within the genus *Gordius* mainly differ in the cuticular fine structure and in cuticular characters in the male posterior end. Fine structural documentation by scanning electron microscopy is the method of choice.

Adult, free living horsehair worms are mostly found in freshwater, where they may occur in all kinds like lakes, ponds, rivers, streams or even artificial water bodies such as cattle troughs. There are few cases, where gordiids were reported outside the water, most prominently in the species *Gordius terrestris* (Anaya et al. 2019). At least in temperate regions, gordiids show a seasonality in their occurrence and emerge from their hosts in late summer (e.g. Schmidt-Rhaesa et al. 2005; Schmidt-Rhaesa and Perissinotto 2025). Here, we report the first record of a gordiid from Pakistan and describe it as a new species from an unusual habitat.

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Material and methods

Worms were collected from two different districts, Dir Upper and Upper Kohistan of Khyber Pakhtunkhwa, Pakistan. In Dir Upper collection was made from two different but closely situated (at about 2.5 km) villages: Gat Koto (35°03'38"N, 71°36'59"E; Fig. 1, type location) (n = 13) and Shaltaloo (35°03'26"N, 71°37'33"E; Fig. 1, location 1) (n = 7) of Shahikot region Tehsil Barawal Bandi, District Dir Upper, Khyber Pakhtunkhwa, Pakistan during three separate visits (19th December 2020; 27st December 2021; 08th March 2024). The two villages are at distances of around 18 km and 20 km from Tehsil Municipal Administration (TMA) Barawal Bandi, District Dir Upper respectively. In Upper Kohistan live specimens (n = 4) were collected from Karang (35°28'43.8"N, 72°57'39.8"E; Fig. 1, location 2) Tehsil Kandia, District Upper Kohistan on 10th January 2025. All the times and at all the localities animals were collected alive and actively wriggling from freshly fallen snow (Fig. 2A, B) early morning. Worms were not found in freshwater even after thorough searches in the locality. Potential hosts were not observed close to the worms. The animals were transported in spring water to the Laboratory of Entomology and Vector-borne Diseases, Institute of Zoological Sciences, University of Peshawar, Khyber Pakhtunkhwa Pakistan. Gross morphological features were studied under a stereomicrocospe (Olympus SD 30 fitted with microscope camera MicroOkular, Bresser GmbH, Germany) from live specimens. After live observation, male specimens were preserved in 70% ethanol while females were reared to check for oviposition and development. Female specimens collected during the 2nd visit were reared in spring water from the natural habitat in plastic bottles until their natural death in the first week of March 2022. After a week preservation in alcohol, mid body fragments were mounted in Puri's gum-chloral medium, which is used for insect clearing and mounting. Females were also preserved in 70% ethanol after death.

For scanning electron microscopy, 0.5 cm fragments of anterior, posterior and mid body regions were treated with 2.5% glutaraldehyde overnight followed by alcohol dehydration (30%, 50%, 70%, 90% and 100% ethanol; 1 hour treatment in each). The fragments were mounted on stubs by double stick carbon tap and coated with gold in a sputter (SPI-Module™ Sputter Coater, SPI Supplies Division of Structure Probe, Inc.). Images were taken with scanning electron microscope (Model: JSM5910, Jeol, Japan) in Centralized Resource Laboratory (CRL), University of Peshawar.

Results

***Gordius nixus* sp. nov.**

https://zoobank.org/56E6741E-1DF9-4A90-B154-79206D02F87A

Type locality. Village Gat Koto (35°03'38"N, 71°36'59"E; Altitude = 2333.12 m) (Fig. 1), Shahikot, Tehsil Barawal Bandi, District Dir Upper, Malakand Division, Khyber Pakhtunkhaw Province, Pakistan. Over freshly fallen snow (Fig. 2A, B), collected on 27th December 2021.

Holotype. Male from type locality, anterior, posterior and mid body segments (1 cm each) as SEM sample, rest of the body preserved in 96% ethanol. Deposited in the Pakistan Museum of Natural History (accession number: PMNH-ZSD-INV-NEMA-0001).

Allotype. Female from type locality, anterior, posterior and mid body segments (1 cm each) as SEM sample, rest of the body preserved in 96% ethanol. Locality of the allotype is the same as that of the holotype (35°03'38"N, 71°36'59"E; Altitude = 2333.12 m). Deposited in the Pakistan Museum of Natural History (accession number: PMNH-ZSD-INV-NEMA-0006).

Paratypes. Anterior, posterior and mid body segments (1 cm each) of two males and one female as SEM sample and rest of the specimens preserved in 96% ethanol. One further male specimen with the anterior end mounted on a slide and the rest preserved in 96% ethanol. All these paratypes belong to the same locality as type locality (35°03'38"N, 71°36'59"E; Altitude = 2333.12 m). Accession numbers for the paratypes are: PMNH-ZSD-INV-NEMA-0002 to PMNH-ZSD-INV-NEMA-0005.

Further material. Fragments of 11 further specimens preserved in 96% ethanol (n = 6 from Gat Koto, 35°03'38"N, 71°36'59"E, the type locality and n = 5 from Shaltaloo, 35°03'26"N, 71°37'33"E) (Fig. 1, type locality and location 1) and 4 specimens from Karang (35°28'43.8"N, 72°57'39.8E District Upper Kohistan; Fig. 1, location 2) as gold coated SEM stubs are in possession of QJ in the Institute of Zoological Sciences, University of Peshawar, Peshawar, Pakistan.

Putative localities of actual spotting on snow and from water. Further confirmed spotting on snow and from water are reported to the authors from the following locations: village Hattan Dara, District Dir Upper Khyber Pakhtunkhwa, Pakistan (35°12'30"N, 71°52'29"E), altitude: 1447.68 m (Fig. 1, location 3); Dir Bazar, District Dir, Upper Khyber Pakhtunkhwa, Pakistan (35°11'30.0"N, 71°52'29.0"E) (Fig. 1, location 4); village Jijal, Tehsil Pattan, District Lower Kohistan Khyber Pakhtunkhwa, Pakistan (35°12'29"N, 71°52'29"E), altitude: Altitude = 1551.11 m (Fig. 1, location 5); village Chatal Kayal Khar, District Lower Kohistan, Khyber Pakhtunkhwa, Pakistan (35°10'08"N, 73°01'34"E), altitude: 1418. 87 m (Fig. 1, location 6); Kakul Road, District Abbotabad, Khyber Pakhtunkhwa, Pakistan (34°10'22"N, 73°14'09"E), altitude: 1223.15 m (Fig. 1, location 7); Mian Kaley Munda, District Lower Dir, Khyber Pakhtunkhwa, Pakistan (34°49'19"N, 71°40'21"E), Altitude = 779.8 m (from rice paddy) (Fig. 1, location 8); Fatehpur, Tehsil Khwazakhela Distrct Swat, Khyber Pakhtunkhwa Pakistan (35°04'10"N, 72°29'11"E), Altitude = 1418. 87 m (from water) (Fig. 1, location 9). These specimens were not investigated morphologically.

Etymology. The word "*nix*" is Latin for snow. The species epithet "*nixus*", therefore, is chosen as the worms were collected over freshly fallen snow.

Description. Male (n = 11); Length/width (n = 04):185 mm/1 mm, 165 mm/1 mm, 110 mm/0.9 mm,

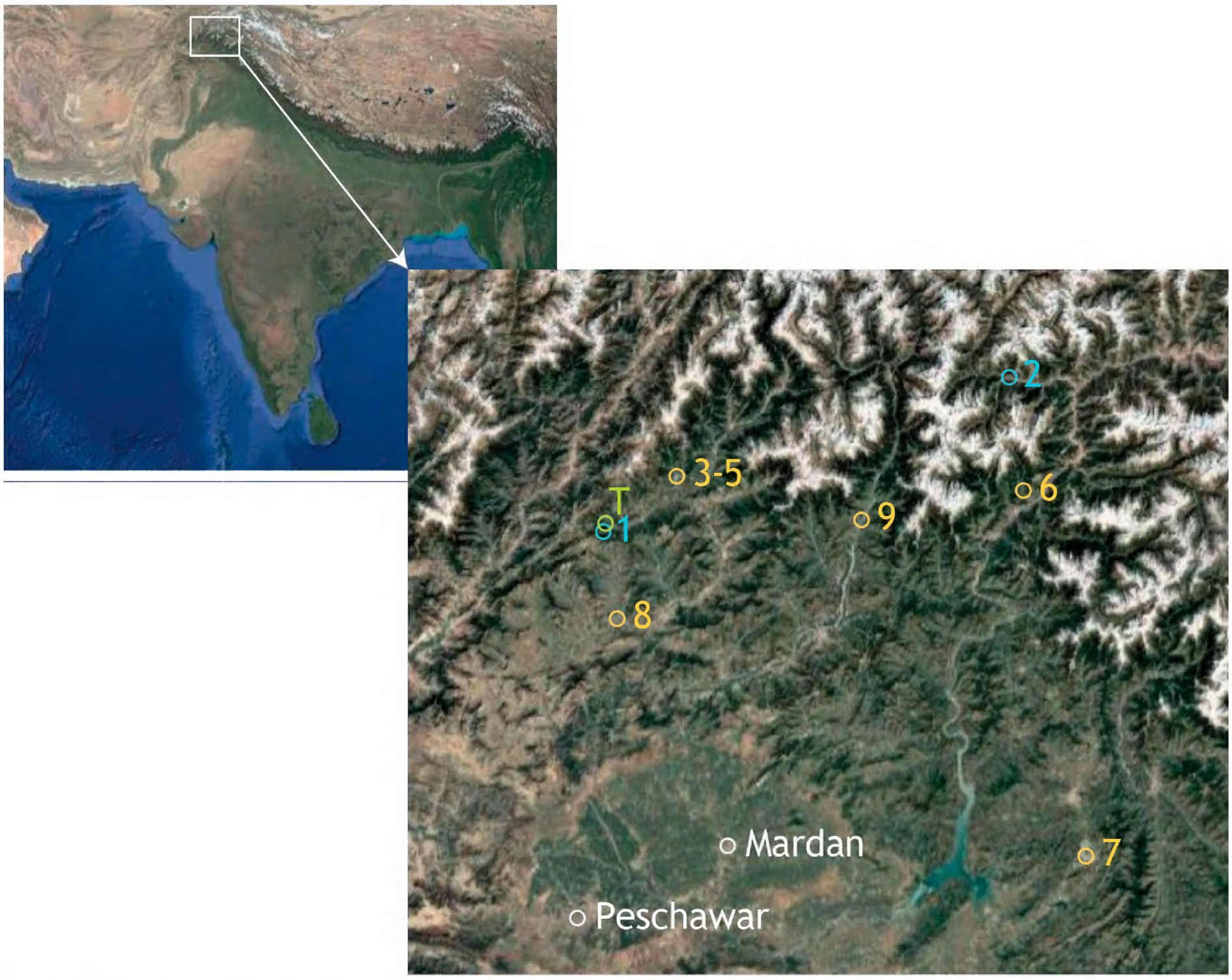

Figure 1. Distribution of *G. nixus* sp. nov. in Hindu Kush, Karakoram and Himalayan ranges. T = type locality (Gat Koto 35°03'38"N, 71°36'59"E). Further locations with investigated worms are labelled in blue (Shaltaloo, 35°03'26"N, 71°37'33"E as location 1 and Karang, 35°28'43.8"N, 72°57'39.8E as location 2). Further locations with confirmed, but not further investigated specimens are: location 3: village Hattan Dara (35°12'30"N, 71°52'29"E), location 4: Dir Bazar (35°11'30.0"N, 71°52'29.0"E), location 5: village Jijal (35°12'29"N, 71°52'29"E), location 6: village Chatal Kayal Khar (35°10'08"N, 73°01'34"E), location 7: Kakul Road, District Abbotabad (34°10'22"N, 73°14'09"E), location 8: Mian Kaley Munda (34°49'19"N, 71°40'21"E), location 9: Fatehpur (35°04'10"N, 72°29'11"E). See text for more extensive description of locations.

183 mm/0.9 mm; color: yellowish brown to light brown when alive, turned dark brown after preservation in 70% ethanol. Live specimens carried light creamy white spots on the cuticle, which were in greater density on anterior to mid-body and became scant towards posterior; spots almost lacking in the posterior region. Two creamy lines, prominent in the anterior, extended along the midline of the body dorsally. Anterior tip with white calotte followed by a dark black collar (Fig. 2D). Posterior end with semi-circular postcloacal crescent, and two blunt lobes (Fig. 2F; length and width measured in two specimens = 0.6 mm/0.32 mm, 0.7 mm/ 0.3 mm). The lobes had depressions on inner ventro-medial side towards proximal end in scanning electron micrographs (Fig. 3E, F). Cuticle surface smooth throughout, sometimes with roundish to polygonal imprints (Fig. 3B). Spines are present in the anterior end (Fig. 3A, C). They showed greater density at the anterior half of the body and decreased towards posterior. Spines in the cloacal region and over the tail lobes were sparsely distributed and short (Fig. 3D–F). The cloacal opening could not be observed very well, as it was either covered by dirt (Fig. 3E) or appeared to be absent (Fig. 3F). The postcloacal crescent is semicircular and directly at the level where the tail lobes divide (Fig. 3D–F).

Female (n = 13); Length/width (n = 05): (160 mm/1 mm, 175 mm/1.1 mm and 255 mm/1 mm, 210 mm/1 mm, 187 mm/1.1 mm); Color: yellowish brown to brown in life (Fig. 2E) and turned dark brown in 70% ethanol. Like males, living females also carried light creamy white spots on the cuticles that were dense on anterior till mid body but became scarce towards posterior. These spots are almost always lacking in the posterior end. Two creamy lines, prominent in anterior region, were running along the dorsal midline of the body. The anterior tip had a white calotte followed by a dark black collar. The anterior region had a dense covering of fine bristles visible in glycerin mounted

Figure 2. A, B. Living specimens on snow; **C.** Abundant bristles along the anterior end; **D.** Anterior end with white calotte followed by a dark ring; **E.** Tangle with several specimens. Some females have sperm on their posterior end (arrows); **F.** Ventral view on the posterior end of a male showing the cloacal opening (co) and the postcloacal crescent (pcc).

10% KOH cleared specimen (Fig. 2C) and scanning electron micrograpy (Fig. 4B–E). Spines become much less abundant and shorter in the middle and posterior body region. The cuticular surface is smooth, as in males (Fig. 4A). Posterior end round, bearing a vase-like cloacal opening at the tip (Fig. 4B). Some females had a sperm drop on their posterior end (Fig. 2E).

Differential diagnosis. The smooth cuticle as well as the morphology of the male and female posterior end correspond to several other species in the genus *Gordius* and do not set the specimens from Pakistan apart from other species. What is unique is the dense presence of spines in the anterior end. Several *Gordius* species have sparsely distributed short spines on the body cuticle (see character 4 in table 1 in Schmidt-Rhaesa 2010), but to our knowledge only in two species bristles were described in the anterior end. In *Gordius terrestris* from North America, there are very fine bristles (see e.g. image 1B in Anaya et al. 2019), in some specimens these seem to be lacking (e.g. image 3B in Analya et al. 2019). *Gordius terrestris* has polygonal areoles on the cuticle, in contrast to the Parkistan specimens. The other species with bristles in the anterior region is *Gordius wulingensis* from China (Zou et al. 2025), but in smaller number than in the specimens from Parkistan. As most diagnostic characters for identifying species in the genus *Gordius* are so far found in the male posterior end, less attention might have been put to the anterior end. Nevertheless, the presence of dense bristles in the anterior end is a unique character and justifies the description as new species.

Remarks. Mid-body segments of adult male fixed in 70% ethanol and mounted in Puri's medium for insect clearing and mounting took a year to become clear enough for microscopy. Female specimens preserved in 70% ethanol for a week when placed in 10% KOH, also an insect and arachnid clearing agent, overnight was completely dissolved leaving behind a shroud cuticle all along body except the anterior collar (calotte was also dissolved) and posterior tip (Fig. 3A inset, B). The collar and posterior cloaca containing tips, however, became nicely cleared for microscopy. This treatment also helped revealing bristles on the cuticle around collar (Fig. 3B). Adult mated females (Fig. 2E with sperm on the posterior end) lived for more than 2 months in spring water when reared in lab from 21st December, 2020 to 5th March 2021 (n = 2) and 29th December, 2021 to 13th March, 2022 (n = 3) at room temperature with average day and night temperature of 20 °C and 5 °C December through February.

Ecological remark. The finding of nematomorphs on snow is quite remarkable. As this was observed repeatedly, we believe that this is a natural occurrence. However, the life cycle of *Gordius nixus* remains completely unknown. No host species was found close to where worms were found. The life cycle of horsehair worms is still not well understood, but at least in temperate regions the life cycle seems to be coupled to seasons. For example in central Europe, free living specimens are found most abundantly in summer and fall and at least in some species there appear to be species-specific time windows of occurrence (e.g. Schmidt-Rhaesa et al. 2005). Adult specimens die after deposition of gametes and nematomorphs might survive the winter either as eggs, larvae or within the intermediate host (e.g. Bolek et al. 2013). There are some occasional records of finding adult nematomorphs during winter and under icy conditions (e.g. in Mühldorf 1913), but such records are rare and seem to be the exception.

The association of *G. nixus* with snow is a new and exceptional phenomenon among nematomophs and deserves further observations concerning seasonality, partner finding and hosts.

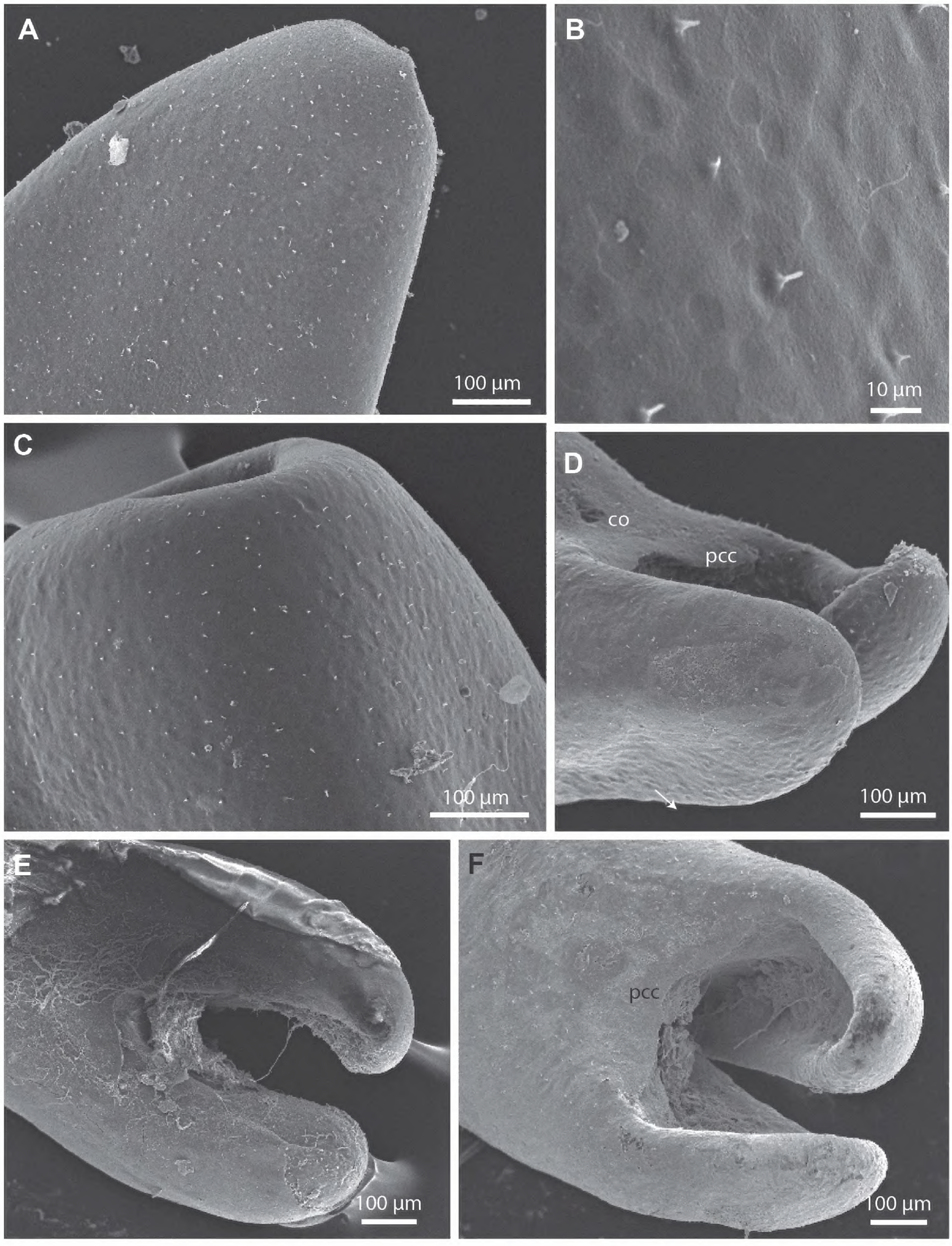

Figure 3. Scanning electron micrographs from male specimens. **A.** Dense bristles on the anterior end of new male 1; **B.** Magnification of bristles and cuticular surface; **C.** Bristles on the anterior end of the holotype; **D–F.** Lateral or ventral views on the posterior ends of several specimens, showing the cloacal opening (co) and the postcloacal crescent (pcc).

Geographic remarks. *Gordius nixus* sp. nov. is the first formal description of a horsehair worm from Pakistan. As we are aware of further, unpublished observations of horsehair worms, we are sure that their diversity and distribution is much higher in Parkistan than currently known.

Like Parkistan, little is known about the Nematomorpha from neighboring countries. There is no record from Afghanistan and only three records (*Chordodes anthophorus*, *C. bipilus*, *Parachordodes* sp.) from Iran, all from the northern region (Kirjanova 1957; Mohtasebi et al. 2021, 2022). From India, a total of 22 species (and some

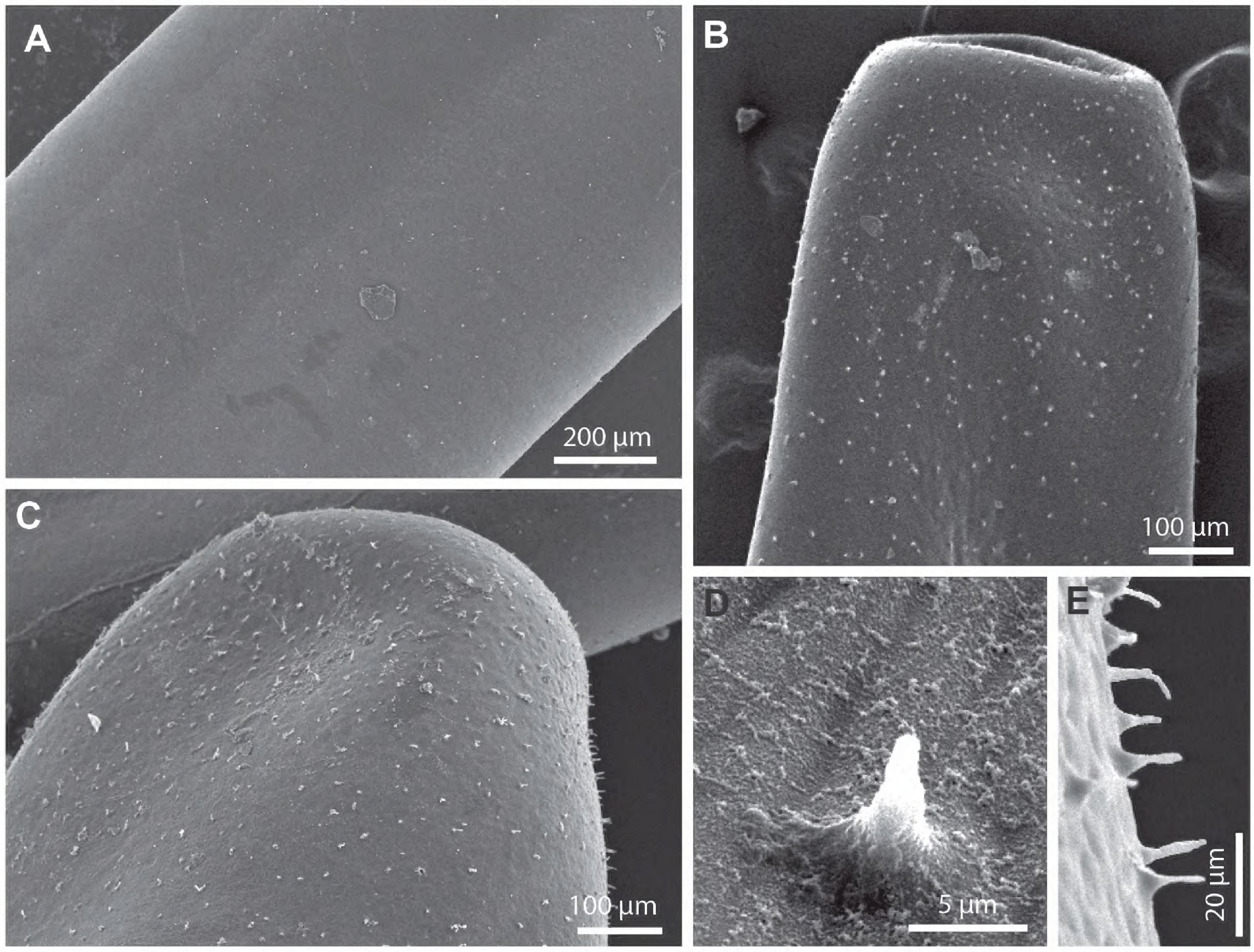

Figure 4. Scanning electron micrographs from female specimens. **A.** Smooth cuticle in female specimen from Kohstan; **B, C.** Dense bristles in the anterior end of allotype (**B**) and female specimen from Kohstan (**C**); **D.** Magnification of bristle; **E.** Lateral view on bristles; **F.** Posterior end with cloacal opening (co) in the female paratype; **G.** Bristles in the anterior end.

further unidentified records) are known, but the majority of them are from Eastern India (Yadav et al. 2021) and only three records come from northwestern regions, namely Punjab (*Paragordius stylosus*, see Camerano 1912) and Uttarakhand (*Acutogordius doriae* and *Parachordodes roccatii*, see Camerano 1908, 1912). From China, 17 species from 5 genera are reported, including 6 *Gordius*-species (Schmidt-Rhaesa 2013). With the exception of *Gordius wulingensis* (Zou et al. 2025), the *Gordius* species are not sufficiently well described to allow an in-depth comparison.

Although part of the region is dry, there are other parts, which have streams and rivers and appear well suited for nematomorphs. Therefore, the biodiversity of horsehair worms in Pakistan as well as in the neighboring regions is expected to be considerably higher than the current records suggest. Hopefully, records such as this one help to motivate the search for horsehair worms and report them.

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